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Heparin-induced thrombocytopenia: Impact of bovine versus porcine heparin in HIT pathogenesis

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1. ABSTRACT

Heparin-induced thrombocytopenia (HIT) is a complication of heparin therapy in cardiovascular/hematologic indications. Heparin is a mixture of sulfated mucopolysaccharide with heterogeneity and is capable of forming multiple complexes with platelet factor 4 (PF4), released from activated platelets. HPF4 antibodies may cause platelet/endothelial cell activation to promote HIT pathogenesis. HIT is a clinico-pathologic syndrome and its diagnosis primarily remains a clinical one; however, the serologic confirmation of the presence of HPF4 antibodies is also necessary part of the evaluation. Assays are based on the immunodetection of HPF4 antibodies and/or their functional ability to activate cells. Currently, there are several assays in use and a few newer/rapid immunoassays are becoming available. Recent studies have confirmed that HPF4 antibody generation (seroconversion) is common after cardiac surgery and suggest that patients receiving bovine heparin are more likely to generate functional (pathogenic) HPF4 antibodies of the IgG subclass. Thus, the use of bovine heparin in cardiovascular surgery should be avoided. A brief account of the currently available options for the management of HIT patients with non-heparin anticoagulants is provided.

2. INTRODUCTION

Heparin, a mysterious agent, is one of the most widely used parenteral drugs in modern medicine. Its use in the treatment and prevention of thromboembolic (TE) disorders is unparallel to any other anticoagulants, with the use of trillions of units worldwide. Beneficial characteristics of heparin therapy (both intravenous as well as subcutaneous) include proven efficacy, rapid onset of action, ease of laboratory monitoring, rapid neutralization, and a significantly lower cost (1). Unfractionated heparin (UFH) is usually manufactured from two major sources of animals, viz., beef lung or pork gut. It remains the anticoagulant of choice for cardiopulmonary bypass surgery (CPBS), despite such potential complications as bleeding and heparin-induced thrombocytopenia (HIT). HIT is most often associated with exposure to UFH, but it may also occur with low-molecular-weight heparins (LMWH); albeit at an approximately 10-fold lower risk (2-4). In the U.S. alone, approximately 12 x 10^6 patients receive heparin annually, translating to approximately 360,000 new HIT cases each year (5).

Immune-mediated HIT (often referred to as HIT type II) is typically characterized by a fall in platelet count

of >50% (with or without thrombosis), usually 5-10 days after exposure to heparin (6-8). The condition is due to the generation of a heterogeneous group of antibodies against the complex formed between heparin and platelet factor 4 (HPF4) molecules, which activate platelets and/or endothelial cells by binding to FcγIIa (CD32) receptors (9-12). Since heparin and related drugs are negatively charged polyelectrolyte chains (mucopolysaccharides) capable of mobilizing PF4 oligomers, the phenomenon is molecular-weight- as well as charge- dependent to cause immunogenicity. Molecular structural and biological studies on the heterogeneity of the HPF4 antibodies support the hypothesis that two main groups of antibodies are generated (seroconverted) during the progression of HIT syndrome, i.e., functional and non-functional HPF4 antibodies (7, 13).

Seroconversion rates vary widely, and may depend on various factors, including the chemical composition of the heparin preparation used, dosage, and the underlying clinical situation of the patient concerned (7, 14). Clinical HIT following cardiovascular surgery is relatively uncommon (< 2%). However, the reported incidence varies according to how thrombocytopenia was defined, and which laboratory assay was used, since this dramatically affects the sensitivity and specificity for clinical HIT (2, 3, 5, 15, 16). Despite the low incidence of symptomatic HIT, HPF4 antibody formation in the absence of HIT occurs in >50% of patients following open-heart surgery (17).

This minireview is organized into two of the many important aspects of HIT syndrome, i.e., a) clinical and laboratory diagnostic features, and b) nature of HPF4 antibody production during anticoagulation with bovine and porcine heparins and its implications in HIT pathogenesis.

3. DIAGNOSIS OF HIT SYNDROME

3.1. Clinical Features

HIT is a "clinicopathologic" syndrome, whereby i) the diagnosis is made most confidently when the patient has an episode of thrombocytopenia that cannot otherwise be readily explained, and ii) together with the presence of HPF4 antibodies that usually give strong positivity using sensitive/specific laboratory assay(s). Clinical recognition of HIT is paramount - the diagnosis of HIT is largely based on clinical findings, i.e., typically a falling platelet count with or without arterial/venous thrombosis in patients currently (or recently) exposed to heparin (5).

The timing of the onset of thrombocytopenia shows three characteristic profiles. The most common, which is observed in up to 70% of patients, is referred to as "typical onset HIT", in which the platelet count begins to fall 5-10 days after starting heparin therapy (18). This characteristic delay reflects the usual short interval for heparin to initiate a humoral immune response. In contrast, the "rapid-onset HIT" is recognized in about 25-30% of patients where platelet count falls abruptly within 24 h of starting heparin therapy. This syndrome result from a previous immunizing exposure to heparin (usually within the past few weeks), and the platelet count falls quickly because the patient already has circulating HIT-associated antibodies when the heparin is re-administered. The third or last syndrome called "delayed-onset HIT", a rare though often clinically serious, is observed in only < 5% of patients and recognized by a fall in the platelet count that begins several days after heparin therapy has been stopped (19). This situation is associated with high titer functional HPF4 antibodies (often referred to as "*superactive*" class of HPF4 antibodies) that do not require exogenous/ongoing heparin administration to exert their pathogenic effects (7, 13, 20). While keeping these facts in mind, the clinicians must evaluate the patient for other potential explanations for the thrombocytopenia, such as perioperative hemodilution, sepsis, multiorgan dysfunction syndrome, immune thrombocytopenia caused by other drugs, and post-transfusion purpura, etc.

Recently, a new scoring system, called the "4 Ts" has been proposed to identify patients with clinical features of HIT (11). Although, not fully validated yet, this clinical scoring system is based on the characteristic features of HIT, including *T*hrombocytopenia, *T*iming, *T*hrombosis, and the absence of o*T*her explanations. The four clinical features (Ts) are assessed and given scores of 0, 1, or 2, thus the maximum total score could reach up to 8. Estimated pre-test probabilities of HIT thereby range from low (score 0-3), an intermediate (score 4-5), and to high risk (score 6-8). The practical implication of the "4 Ts" scoring system would be to help clinicians make initial decision regarding alternative anticoagulant therapy required by HIT patients on heparin.

3.2. Laboratory Features

HIT-associated antibody detection is rapidly becoming a standard of care in hematology and cardiology. Two main class of laboratory tests have thus far been categorized, i) functional, and ii) immunologic (also referred to as non-functional), each with its own advantages and disadvantages. Currently available laboratory tests for the diagnosis of HIT are not "ideal", i.e., they are not 100% sensitive or specific. Although, these laboratory tests do provide subsequent confirmation or exclusion of the diagnosis (of clinically suspected HIT), and should therefore, be performed. To adequately evaluate patients, hematologists and other clinicians should know which tests are locally available to them, understand the predictive value of each test, and weigh the clinical value of test results in light of the pre-test probability of HIT. If at all possible, both immunologic and functional (platelet activating) laboratory tests should be performed to confirm the HIT diagnosis.

3.2.1. Functional Tests for HIT

The functional tests (mainly the serotonin release, platelet aggregation, and flow cytometric assays) take advantage of the ability of HPF4 antibodies to activate cells (mainly platelets) in the presence of therapeutic amounts of heparin. By virtue of platelet activation response due to HPF4 antibodies, functional assays determine end-points based directly on the pathophysiology of HIT. All these

tests require a source of normal donor platelets and are performed with either washed platelets (greatly preferred) or platelet-rich plasma of the donor. The ^{14}C-serotonin release assay (SRA) is the most preferred test among all the functional assays due to its high degree of diagnostic sensitivity and specificity (15, 21). Furthermore, in this test, using special buffer conditions and performing the assay in microtiter wells permits the simultaneous examination of numerous reaction conditions, thus maximizing the sensitivity (14).

Despite better laboratory diagnostic test results, the major limitations of these functional assays are their labor-intensive, technically demanding nature and associated high cost, and therefore some of the reference laboratories, particularly in developing countries, are unable to afford it. Additionally, freshly prepared normal donor platelets are pre-requisite for each batch of assays, and all potential donors may not give similar reproducible responses on a day-to-day basis. In fact, only about 40% of all the potential healthy donors are reactive in the functional tests, and that's why these assays are poorly standardized between the laboratories. Also, these assays are not well suited for testing large volumes of samples, forcing many reference laboratories to rely on immunoassays. To overcome some of these problems/limitations, newer functional tests for HIT diagnosis at near-bedside, such as thrombelastograph (TEG), etc., are currently under development, where donor's fresh whole blood could be utilized to assess the global clotting parameters that may provide rapid and more clinically relevant information about the HPF4 antibody characteristics in a given patient specimen.

3.2.2. Immunoassays for HIT

The most commonly used tests for the laboratory diagnosis of HIT are solid-phase HPF4 antibody immunoassays. These immunologic tests directly demonstrate antibody binding to the PF4 and polyanion (e.g., heparin) complex, and therefore, merely confirm the presence of the antibody (telling no account of its ability to cause functional responses). These tests include the ELISA, the particle gel immunoassay (PaGIA), and flow cytometery for antigen binding. Currently, two types of ELISAs are commercially available, which are approved by the U.S. Food and Drug Administration (FDA), and both of which detect the antibodies of the three major Ig classes (i.e., IgA, IgG, and IgM) against PF4 bound either to heparin (Asserachrome HIPA, Diagnostica Stago, France) or polyvinyl sulfonate (Genetic Testing Institute; GTI, Inc., Waukesha, WI). The Stago assay utilizes recombinant PF4, whereas the GTI obtains the PF4 from outdated platelets. In these assays, positive results are indicated by an optical density (OD) value above the pre-determined threshold. Many laboratories simply report results as positive or negative; however, recent reports suggest that the higher antibody titers (OD values) in a given patient may have more clinical significance towards causing the HIT pathogenesis (22, 23). Using these ELISAs, some research laboratories have the option to detect only a specific class of antibodies (e.g., IgG), which increases specificity for clinical HIT by avoiding the detection of IgA and/or IgM subclasses (largely non-pathogenic in HIT context) (24-26).

Emerging evidence suggests that knowledge of the patient's actual HPF4 antibody titer (OD value) may be helpful in interpreting the test results (23). For a number of years, we observed a close correlation between the degree of serotonin release (in the functional SRA test) and the ELISA OD. In fact, most often high OD values (>1.0) are much more likely to be associated with a positive functional test result. Chilver-Stainer et al (22) recently reported that HPF4 antibody titer as measured by commercial ELISAs and other emerging rapid test correlated with the level of circulating thrombin-antithrombin (TAT) complex, indicating an association between higher levels of HPF4 antibodies and thrombin generation. This may well correlate with antibody-mediated platelet activation assays leading to higher clinical probability of HIT. Although ELISA is widely used, it is still relatively expensive, and may not be even readily affordable in many developing countries.

A new cost-effective and highly sensitive/specific immunoassay for HIT diagnosis, known as ZYMUTEST HIA (Aniara Corp./HYPHEN BioMed, Mason, OH) is becoming available by as early as December 2006 for evaluation. This assay claims to measure HPF4 antibodies by reproducing the mechanism matching closest to the HIT pathogenesis. Options such as measuring only IgG subclass or to proceed with a full subclasses (IgA/G/M) of HPF4 will be available.

3.2.3. Emergence of Rapid Tests for HIT

Currently available laboratory tests for HIT diagnosis are classified as relatively high complexity, take many hours to perform, and often provide confirmation of HIT or HITTS (HIT associated with thrombosis syndrome) after the symptoms are seen in a patient. In view of this, there has been a need for an easily performed, rapid test that may help clinicians identify and treat patients at risk for HIT / HITTS (27, 28). Of late, a couple of rapid antigen assays for the detection of HIT-associated antibodies has emerged. The first one is a PaGIA test (DiaMed, Inc., Basel, Switzerland) - currently available only in Australia, Canada, Europe, and New Zealand, etc. - directly and rapidly demonstrate the presence of HPF4 antibodies (turn-around-time only 15-20 min). This test uses colored, high-density polymer beads coated with the HPF4 antigen, and provides a qualitative result (positive or negative). In this PaGIA test, the patient's serum is mixed with a suspension of the antigen-coated beads (microspheres), and the mixture is subsequently introduced to a gel that has pores of a fixed size. HPF4 antibodies (if present) in the test sample bind and cross-link the antigen-coated beads, producing a complex that is physically too large to pass through the gel. Thus, the degree of migration of the pink-colored bead suspension through the gel matrix indicates the presence or absence of HPF4 antibodies. Additionally, these HPF4-coated polymer beads are now being utilized in some laboratories for a simple and relatively rapid flow cytometry assay, where the beads are exposed to patient's serum – binding of HPF4 antibodies can be readily detected

with fluorescent dye conjugated to human anti-IgG. Preliminary results from our laboratory using flow cytometry technique appear to correlate well with ELISAs (Ahmad S, et al; unpublished observations).

Another new rapid test is the Particle gel Immuno-Filtration Assay (PIFA; Akers Biosciences, Thoroughfare, NJ), is a qualitative *in vitro* diagnostic device designed for the detection of antibodies to PF4 complexed to linear polyanoinoc compounds, and perhaps is the most rapid HPF4 test (results often available within 5 minutes or so). In this PIFA test, dyed microparticles coated with purified PF4 derived from platelet-rich plasma provide the visual signal for the assay results. The ability of matrixed or non-matrixed particles to move through a filter medium is the measure of the reactivity / non-reactivity of the test sample. This manual assay is FDA-approved, and is commercially available mainly in North America.

It is, however, to be emphasized that both of these rapid tests recommend the use of serum samples as oppose to other tests where both plasma and serum, or possibly other biological fluids are readily used.

Regardless of their turn-around-time or cost-effectiveness, all the antigen (immunologic) tests are considered technically simpler to perform than the functional assays, and of course the ELISA can be readily automated. Antigen tests can be batched and performed in large test volumes, and being commercially available, and perhaps widely standardized between the laboratories.

4. IMPACT OF BOVINE AND PORCINE HEPARIN ON HIT PATHOGENESIS

Previous comparative studies, mainly on medical and orthopedic surgical patients, have suggested that bovine heparin is more likely to cause HIT than the porcine product (29-32). These studies essentially included episodes of clinical features (e.g., thrombocytopenia) as no good laboratory tests for the diagnosis of HIT were available during 1970s and 1980s. Indeed, heparin derived from porcine mucosal intestinal became somewhat preferred at many hospitals despite some cardiac surgery units continue to use bovine lung heparin preparation due to a relatively lower risk of post-operative bleeding complications (30, 33). Later on, with the increasing recognition of HIT as an immune-mediated syndrome coupled with the advancement in the development of sensitive/specific laboratory diagnostic tests (both immunologic and functional), the use of bovine heparin remained in question.

Recent study from our institution reported the high frequency of ELISA-detectable HPF4 antibodies after coronary artery bypass graft (CABG) surgery, and demonstrated that bovine heparin was associated with a significantly higher incidence of HPF4 antibody generation (seroconversion) as detectable by ELISA only (34). However, the implications of bovine heparin for clinical HIT in cardiovascular surgery are not clear, as there are no comparative data on the ability of bovine and porcine heparins to generate functional (platelet-activating) HPF4 antibodies following CABG (35). Some of our recent investigations related to the characterization of HPF4 antibodies in terms of functionality (i.e., platelet-activating ability) and immunoglobulin subtypes (IgA, IgG, or IgM) in CABG patients randomized to bovine or porcine heparin is therefore described below.

The original study (34) enrolled 207 patients undergoing first time CABG with or without cardiopulmonary bypass (CPB). Blood samples were collected pre-operatively and daily until discharge or for 7 days, whichever occurred earlier. For the present sub-study, a total of 962 plasma samples from 141 patients (almost equal ratio for the bovine and porcine groups) were available for analyses. Anticoagulation with both bovine and porcine heparin resulted in the generation of HPF4 antibodies (mainly on days 5-7), although the incidence of ELISA positivity was significantly higher following bovine heparin (34). Of these 141 patients, 66 (47%) patients tested positive by ELISA. When these ELISA-positive patients (n=66; 41 in bovine and 25 in porcine groups) were tested by SRA, only 27 (representing 41% of those positive by ELISA, and 19% of the total 141 patients), were functionally active. The proportion of ELISA-positive plasma samples that were also positive by SRA was significantly higher in patients receiving bovine heparin (50%) for CABG compared to porcine heparin (32%; $p<0.01$).

Table 1 compares the number of plasma samples positive by ELISA and SRA in the post-CABG period (days 1-7). As expected, most of the positive results in both assays occurred between 4 and 7 days after heparin exposure. Table 2 compares the number of patients that were antibody-positive by SRA in patients classified according to whether they received bovine and porcine heparin for CABG. Functional (i.e., SRA-positive) HPF4 antibody generation had a similar temporal relationship to the date of surgery, but the number of patients developing functional antibodies was higher at each time point in patients receiving bovine heparin ($p<0.001$). This difference was particularly marked in samples collected on post-operative days 6 and 7. Furthermore, the correlation between the ELISA OD and percentage serotonin release SRA was significantly better ($p<0.001$) in samples obtained from patients receiving bovine heparin (R=0.522) compared to porcine heparin (R=0.269).

These observations therefore confirmed that HPF4 antibodies are common after cardiovascular surgery, and that most patients develop antibodies that are only detectable by ELISA. Notably, this work also demonstrated that patients receiving bovine heparin are significantly more likely to generate functional (SRA-positive), and therefore potentially pathogenic, HPF4 antibodies.

The distribution of immunoglobulin subclass results in all the ELISA-positive samples that were tested by SRA is shown in Table 3. The prevalence of the IgG

Table 1. Frequency of ELISA and SRA positivity for heparin-platelet factor 4 (HPF4) antibodies in post-cardiac surgery patients

Day of Testing	ELISA Positive (n)	SRA Positive (n)
1	4	0
2	4	0
3	10	3
4	16	3
5	37	10
6	41	11
7	26	11

Data represents a day-to-day analysis of the GTI-ELISA vs. ^{14}C-serotonin release assay (SRA) positive responses in seroconverted samples irrespective of bovine vs. porcine heparin group [obtained from among the total of 962 samples from 141 HIT suspected coronary artery bypass graft (CABG) patients].

Table 2. Differential prevalence of functional HPF4 antibodies in patients anticoagulated with bovine or porcine heparin for cardiac surgery

Day of Testing	Total SRA Positive (n)	SRA Positive (Bovine Group)	SRA Positive (Porcine Group)
1	0	0	0
2	0	0	0
3	3	2	1
4	3	0	3
5	10	6	4
6	11	9	2
7	11	10	1

Data represents a day-to-day analysis of the ^{14}C-serotonin release assay (SRA) positive responses in post-coronary artery bypass graft (CABG) patient plasmas obtained from bovine and porcine heparin group (pre-determined to be ELISA positive for heparin-platelet factor 4 [HPF4] antibodies).

Table 3. Distribution of HPF4 antibody subclass in plasmas obtained from post-cardiac surgery patients receiving bovine or porcine heparin for anticoagulation

Ig Subclass	Bovine Heparin Group		Porcine Heparin Group	
	% SRA Positive	*% SRA Negative*	*% SRA Positive*	*% SRA Negative*
IgA	47	32	25	3
IgG	68	32	38	12
IgM	80	63	88	82

Results of heparin-platelet factor 4 (HPF4) antibody subclasses in the GTI-ELISA positive plasmas (n=138) obtained from patients (n=141) receiving bovine or porcine heparin compared to their reactivity in ^{14}C-serotonin release assay (SRA).

subclass of HPF4 antibodies among SRA-positive samples was 2-3 fold higher in both bovine and porcine treated patients. Furthermore, the percentage of patients with a predominant IgG component was higher in patients that received bovine heparin, although this did not achieve statistical significance. Differences in the prevalence of the IgA and IgM subclasses were less marked. This observation is consistent with the fact that binding of IgG antibodies to platelets, regardless of their target specificity, generally induces platelet activation through the FcγIIa receptors (36, 37). In contrast, IgA antibodies expose and interact with FcαR, while the IgM subtypes expose FcμR receptors, which are typically on other cells such as neutrophils, monocytes and lymphocytes.

The reason for the higher frequency of HPF4 antibodies of the IgG subclass among the SRA-positive patients receiving bovine heparin is not clear. It is widely believed that the affinity of different heparin preparations for PF4 could result in varying degrees of antigenicity (38). Hence, it may partly be due to the differences in mobilization of PF4 (formation of large multimolecular complexes that compose the target antigen for HIT-associated antibodies) by bovine heparin, possibly because of its higher molecular weight and increased degree of sulfation (anionic charge density) (32, 39, 40). It has also been suggested that due to its higher molecular weight, bovine heparin has greater affinity for binding to antithrombin and are extremely potent in prolonging the activated partial thromboplastin time (aPTT) and the whole blood clotting time, and thus is more reactive with platelets *in vivo* (40, 41). Bovine heparin also induces greater fibrinogen binding to ADP-treated platelets, compared to other heparin preparations (29, 42). This increased binding in platelets may also be partly due to the higher sulfate:disaccharide ratio in bovine heparin (32, 41, 43) and therefore may be associated with the hyperaggregability seen during heparin therapy. In contrast, the porcine heparin (being relatively lower molecular-weight species) is notably more potent inhibitors of factor Xa than the bovine heparin preparation. Furthermore, lung tissue is reported to have a relatively higher concentration of tissue thromboplastin than that of the intestinal mucosa, and has also been suggested to contain very high levels of van Willebrand factor (44).

Prior to the availability of good HPF4 antibody test systems, Bailey et al (32) suggested that impurities or degradation products associated with the manufacture of bovine lung heparin may be responsible for the higher incidence of thrombocytopenia (29, 45-47). In 1981, the principal manufacturer of bovine lung heparin (Upjohn, Kalamazoo, MI) changed its manufacturing process from an extraction from defatted and dehydrogenated beef lung, after preliminary decomposition, to direct extraction from fresh frozen beef lung tissue (32). Thus, we speculate that its persistently distinct biochemical properties including the higher molecular weight and degree of sulfation are primarily responsible for its greater antigenicity, and therefore increased likelihood of generating functional HPF4 antibodies.

5. MANAGEMENT OF PATIENTS WITH HIT

Despite recent therapeutic advances with LMWH, direct thrombin inhibitors (DTI), factor-Xa inhibitors, and adjunctive therapies, bleeding remains a significant problem in the management of patients with thromboembolic complications. Patients with HIT syndrome can strategically be managed using newer antithrombotic agents that do not produce antibodies against the HPF4 or related complex. The ultimate goal for

managing HIT is to reduce thrombotic risk by minimizing platelet activation and thrombogenesis. Thus, early diagnosis is the key where focus should be on high clinical awareness coupled with best possible serologic screening. If HIT is clinically manifested, all sources of heparin therapy must be discontinued immediately (even in the absence of a confirmatory serological test result), and alternative anticoagulation should be initiated/substituted with approved agents (11, 48). The decision to initiate alternative (non-heparin) anticoagulant therapy should be guided by the assessment of a given individual patient's bleeding risk *vs*. benefit ratio and coexisting/underlying conditions.

Currently there are several alternative anticoagulants available worldwide, *viz*., DTIs (e.g., argatroban, hirudin, bivalirudin) and factor-Xa inhibitors (e.g., pentasaccharide, danaparoid) (49, 50). Most of these agents are approved by regulatory agencies while a few are in the process of further evaluation requiring approval (depending on the clinical observations and the individual country's situations). The DTIs have the advantage of being shorter half-life agents, reversible, show no significant cross-reactivity to heparin, and directly bind and inactivate both circulating and clot-bound thrombin (49, 51). There is no antidote available yet to reverse the effects of DTIs. A recent investigation utilizing TEG system has implicated that argatroban in combination with hydroxyethyl starch could significantly decrease hemostasis (particularly clot propagation and strength) more than argatroban alone (52). If validated clinically, this may potentially be a promising and safer therapeutic approach for managing HIT patients with cardiac surgery where high-dose anticoagulation is required.

Danaparoid is a mixture of heparan sulfate and dermatan sulfate, which catalyzes the AT-mediated inhibition of activated factor X. Although this agent has been used successfully in various non-U.S. countries, a recent report suggests that it can also cross-react with HIT antibodies (53). Pentasaccharide (*aka* fondaparinux) is a FDA-approved, synthetic selective factor Xa inhibitor, which does not cross-react with HIT antibodies (54). Emerging evidence suggest that this agent can be successfully used for the treatment of HIT patients (50).

Long-term anticoagulation with the age-old and cost-effective monotherapy (warfarin; *aka* coumadin) in active HIT patients is contraindicated (55). It is an anticoagulant that acts by reducing the synthesis of the vitamin-K-dependent clotting factors, including factors II, VII, IX and X, and proteins C and S. This agent should not be used alone for the treatment of active HIT; however, it can be used cautiously provided some necessary steps are taken into considerations during the transition from DTI, i.e., continue the DTI alone until the platelet count has returned to >150,000/μL or to pre-test levels, overlap the DTI with warfarin for at least 3-5 days, start warfarin with the expected daily dose (a loading dosage should not be used); and ensure that the international normalized ratio is within the therapeutic range before discontinuing the DTI.

Although the incidence of HIT is about 10-times lower with LMWH than UFH, the use of LMWH should be avoided (at least until the platelet count has recovered and the absence of cross-reactivity of antibody has been demonstrated by an *in vitro* test) (3, 4, 7). Platelet transfusion is contraindicated because it may worsen the potential thrombotic picture.

Additionally, based on theoretical/experimental possibilities, there are some adjunctive therapies that are often practiced in limited or select situations to manage HIT patients. These include: ancrod (*aka* Arvin; a defibrinogenating agent that cleaves fibrinopeptide A from fibrinogen), medical thrombolysis (e.g., use of streptokinase, tissue plasminogen activator) (56), surgical thromboembolectomy, intravenous gammaglobulin (both intact IgG as well as Fc fragments), plasmapheresis, use of immunosuppressive agents (e.g., cyclosporine), defibrotide (a mammalian single stranded DNA-derived antithrombotic/anti-ischemic agent, which does not produce systemic anticoagulation) (57), and the use of antiplatelet agents (e.g. GPIIb/IIIa receptor antagonists, aspirin, dipyramole, etc) (12, 58).

Thus, in an ideal world, the best strategy would be to do clinical trials comparing all the above noted agents with one another to find meaningful answer(s) for managing HIT patients, which unfortunately is near-impossible because of the differences in study design, patients population, and the relative safety/efficacy profile and mechanism of action(s) of individual agents to achieve therapeutic target. Recommendations for their use in the treatment of HIT syndrome are essentially based on prospective and historically controlled clinical studies. Hence, the choice of alternative anticoagulant/antithrombotic therapy to manage HIT should be tailored according to the specific need/condition of a given patient, available resources, and reasonable consensus guidelines.

6. SUMMARY AND PERSPECTIVES

HIT is a serious, immune-related complication of heparin therapy, often resulting in devastating TE outcomes. Advances in the diagnosis and treatment of HIT syndrome in the past few years have led to incremental awareness. Among individuals exposed to heparin, depending on the patient population, about 7-50% develops HPF4 antibodies and about 1-5% develops clinical HIT. The number of patients exposed to heparin is rather large, including not only those undergoing cardiac/orthopedic procedures and VDT prophylaxis or treatment, but also patients having heparin flushes or heparin-coated catheters in many cardiovascular/hematologic patients. Unfortunately, HIT remains fairly under-recognized and under-diagnosed, often with devastating TE complications. Continued educational initiatives are needed to promote its recognition, prompt diagnosis and appropriate treatment (8).

HIT is a clinicopathologic syndrome and its diagnosis primarily remains a clinical one; however, the

serologic confirmation is also a necessary part of the evaluation. The demonstration of antibodies directed against the HPF4 complex is one of the most important components of the laboratory diagnosis of HIT. Commercially used laboratory assays for HIT are ELISAs and rapid tests devices that measure the antibody against HPF4 complex. Functional (platelet activation) assays are gaining more recognition as it closely relates with the immunobiology of HIT pathogenesis. Regardless of the specificity/sensitivity magnitude of a given test, the HIT antibody test results should be interpreted in the appropriate context of the available clinical information of the patient. It is hoped that the transient nature of HIT antibodies and the role of immune memory in HIT will be elucidated, allowing refined identification of "at risk" patients who would benefit from alternative anticoagulation and treatment.

Recent studies from out laboratory clearly suggest that there is a higher frequency of functionally active HPF4 antibodies of the IgG subclass in patients receiving bovine lung heparin for cardiovascular surgery. Although the frequency of clinical HIT was rather low, the data suggest that the use of bovine heparin in cardiac surgery would increase the risk of this complication. The use of bovine heparin for cardiac surgery should therefore be avoided. Of course, further characterization of HPF4 antibodies and profiling of various surrogate markers of hemostasis (as risk factors) is likely to provide a better understanding of the observed differential seroconversion rate and functionality of HPF4 antibodies following anticoagulation with bovine lung or porcine gut heparin.

Finally, to manage HIT patients, heparin cessation alone is inadequate treatment - rather alternative anticoagulation must be initiated to combat the ongoing "platelet activation and thrombin storm" during symptomatic HIT. Currently there are several alternative non-heparin anticoagulants available worldwide, and a variety of them are approved by regulatory agencies while a few are in the process of further evaluation/approval. Obviously, the choice of alternative anticoagulant/antithrombotic therapy to manage HIT should be tailored according to the specific need/condition of a given patient, available resources, and reasonable consensus guidelines.

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Key Words: Heparin-Induced Thrombocytopenia, Diagnosis, Management, Treatment, Heparin-Platelet Factor 4 Antibodies, Bovine *vs*. Porcine Heparin, Cardiac Surgery, Platelet Activation, Immunoglobulin Subtypes, Pathophysiology, Thrombosis, Review

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